foods we used included 9 commercial foods and 2 gelatin-bound formulations that we prepared. Concurrent studies on food particle size suggest that juvenile shrimp prefer large food particles which they can hold and break pieces from rather than small particles that can be ingested whole.

We also directed research toward the problem of bringing penaeid shrimp into spawning condition in the laboratory. Adult shrimp, held in four ponds and several large tanks inside the laboratory, were fed different diets and observed for sexual development. Only males in ponds showed apparent signs (externally) of sexual maturation.

We added several new research facilities and modified others this past year. Twelve concrete tanks, 3 m. long, 1 m. wide, and 1 m. deep (10 ft. by 3 ft. by 3 ft.) were constructed adjacent to our East Lagoon Laboratory. These tanks are connected to the Laboratory's sea-water system and are covered by an open-sided shed roofed with fiberglass. A heat exchanger was incorporated into the sea-water system at Fort Crockett. A 7,700-liter (2,100-gallon) tank, six 1,850-liter (500-gallon) tanks, and equipment for regulating water temperature in the tanks were installed in the East Lagoon Laboratory. We now have a pellet maker that we can use to make experimental foods in sufficient quantity for large-scale experimentation. We made many modifications to facilitate handling and care of larval shrimp, and installed a light room for the mass culture of algae. In addition, a contract was let to the U.S. Army Corps of Engineers for a design to convert the basement of a demolished building into a recirculating sea-water system.

Personnel in the Florida Bay Ecology Studies Project prepared manuscripts that summarized the results of past research. Following the recommendation of the budget review committee, we terminated this project at the end of the year and transferred the personnel to the Bureau's TABL (Tropical Atlantic Biological Laboratory), Miami, Fla.

Harry L. Cook, Program Leader

Larval Culture

We concentrated during the year on determining the physiological requirements of shrimp larvae and developing a prototype hatchery for the culture of larval shrimp. To supply food for the larval shrimp, we also spent considerable time developing a method to grow mass cultures of algae in artificial media.

Larval physiology.--As the result of feeding experiments completed during the year, we concluded that Thalassiosira sp. is the most suitable alga of those tested as food for the larval shrimp. Other algae tested were Skeletonema costatum, Cerataulina sp., Cyclotella nana, and Isochrysis galbana. Table 2 shows the results of one experiment in which brown shrimp protozoeae were fed Thalassiosira. Further analysis of these data showed that protozoeae I survived best when Thalassiosira was supplied at rates of 180,000 to 190,000 cells per larva per day; protozoeae II at rates of 240,000 to 290,000 cells; and protozoeae III at 340,000 to 370,000 cells. In another experiment myses of brown shrimp were fed Artemia nauplii and Thalassiosira. Those animals fed a mixture of Artemia and algae grew faster than those fed only Artemia, but survival was similar except when the numbers of Artemia nauplii supplied were too low.

Table 2.--Concentrations of Thalassiosira supporting the best survival of brown shrimp protozoeae, Biological Laboratory, Galveston, Tex.

Larval stage	Shrimp	Thalassiosira
	Number per liter	Number per ml. (X 1,000)
Protozoea I[1].....	133	30
	167	40
	266	50
	333	60
Protozoea II[2]....	133	40
	167	50
	266	60
Protozoea III[3]...	133	50
	167	60

[1] First-stage protozoea.
[2] Second-stage protozoea.
[3] Third-stage protozoea.

We performed two experiments to determine the effects of salinity on the growth and survival of larvae of brown shrimp. All larval stages in these experiments were held at salinities of 24, 28, 30, and 34 p.p.t. (parts per thousand). EDTA was added to the water in which a portion of the shrimp were held. Survival was best at 28 and 30 p.p.t. Variable survival at 34 p.p.t. indicated that the salinity level probably was near the upper limits of tolerance. Larvae survived well at 24 p.p.t. with EDTA, but they suffered complete mortalities when EDTA was absent. EDTA benefited all stages of larvae at all salinities.

A further test of the effect of EDTA involved the use of a synthetic sea water. Protozoeae III were placed in synthetic sea water (30 p.p.t.) with and without EDTA. Survival was similar in the two groups, but the larvae developed fastest in the water with EDTA.

Mass culture.--Work was directed primarily to the development of a prototype shrimp hatchery. We modified equipment, incorporated

new procedures, and developed new techniques. We modified the larval rearing tanks by adding bulkhead fittings and valves to the bottom of each tank. This arrangement and the addition of a filter screen, which threads into the bulkhead fitting, make it possible to filter, drain, or recirculate the water by opening or closing two valves (fig. 2). A high-pressure power washer (500 pounds per square inch) now used for cleaning equipment in the hatchery enables us to wash and rinse a tank in minutes.

Procedures were developed for estimating numbers of food organisms and larval shrimp. We use a hemocytometer to count diatoms and an electronic counter for first- and second-stage nauplii of the larval shrimp as well as Artemia nauplii. To estimate numbers of more advanced shrimp larvae and postlarvae, we photograph aliquot samples that we place in a petri dish. These pictures are then placed under a dissecting scope and the shrimp counted. We calculate the mean, standard deviation, mortality rate, and confidence limits of the population from these counts.

Gravid white and pink shrimp that we captured offshore spawned successfully in the laboratory. Spawning occurred in the 950-liter (250-gallon) rearing tanks and several 19-liter (5-gallon) carboys. After a female spawned in a carboy, we removed her and put the eggs in the large rearing tanks. We fed the diatom Cyclotella nana to the protozoeal stages and Artemia to the mysis stages. Survival from protozoea to mysis was poor for both species. Although we increased the amount of diatoms fed to the shrimp from 30,000 cells per milliliter to 750,000 cells per milliliter, shrimp larvae continued to die. Only 11 percent of the pink shrimp and 18 percent of the white shrimp survived. We attributed this poor success directly to the diatom Cyclotella, which is an unsuitable food when used alone.

The successful hatching of white shrimp was no doubt due to the fact that their ovaries were fully ripe. Their eggs were a dark olive green. We examined each female and found no attached spermatophore. Water temperature in the spawning tanks were 26.9° to 28.2° C. (80.4° - 82.8° F.), and the salinity 29.1 to 31.2 p.p.t. EDTA at a level of 0.1 g. per liter (0.01 ounce per gallon) was placed in each tank and carboy before the shrimp spawned.

Diatoms are now cultured in artificial sea water in 300-liter (79-gallon) tanks in a temperature-controlled light room. Banks of fluorescent lamps that can be raised or lowered were constructed to provide the required intensity of light. At present, electric pumps transfer the diatoms to the harvesting tanks; however, construction is underway to provide space in the attic where the algae culture room can be transferred. From the attic the algae will be able to flow by gravity to the rearing tanks.

Algal culture.--The diatom, Cyclotella nana, was grown for shrimp food in carboy cultures and in mass cultures with Instant Ocean[1] artificial sea salt (mixed in tap water and prepared without heat) as the basic culture media. To this salt water we added Tris buffer, EDTA, KNO_3, Na_2SiO_3, vitamins B-12 and thiamin, and iron, either as $FeCl_3$ or $FeNH_4(SO_4)_2$. We counted 3 to 6 million diatom cells per milliliter. After the number of diatoms reached a peak (usually 2 days), we harvested about two-thirds of the volume daily and replaced it with new medium to maintain the quality of the culture. We maintained cultures for as long as 14 days.

We studied how artificial sea-salt media would be affected by adding three sources of iron--ferric sequestrine, ferric ammonium sulfate, and ferric chloride. Ferric sequestrine and ferric ammonium sulfate supported good growth of Skeletonema sp. in test-tube cultures, whereas ferric ammonium sulfate supported the best growth of Thalassiosira sp. in tube and carboy cultures.

General suitability of the Instant Ocean-tap water medium for certain flagellates, dinoflagellates, and diatoms in tube cultures was indicated by good growth of 19 to 22 organisms tested.

Harry L. Cook, Project Leader
Ausbon Brown
Cornelius R. Mock
M. Alice Murphy

Food and Experimental Environments

The purpose of our research has been to find a suitable food for raising shrimp. Once we have accomplished this, we can turn our energies to selective breeding. We studied the growth of shrimp fed various foods prepared in our laboratory and fed commercially available foods. We have also completed studies to determine if shrimp prefer certain sizes of food particles.

We continued to use the fish protein concentrate pellet developed last year and modified it to test additional proteins. Pellets were made with fish protein concentrate, cottonseed meal, and soybean meal as the source of protein, either singly or in combination. Test shrimp ate pellets made with fish protein concentrate but not pellets made only with cottonseed meal or soybean meal. The animals tore apart the pellets containing fish and vegetable meals and appeared to extract as much of the fish protein concentrate as possible. A great portion of the vegetable material was left on the bottom of the tank. All experiments had negligible growth and poor survival, apparently from a heavy growth of micro-organisms, fouling of the water, or a combination of these factors.

[1] Trade names referred to in this publication do not imply endorsement of commercial products.